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Central infusions of leptin and GLP-1-(7–36) amide differentially stimulate c-FLI in the rat brain

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Van Dijk, Gertjan, Todd E. Thiele, Jamie C. K. Donahey, L. Arthur Campfield, Francoise J. Smith, Paul Burn, Ilene L. Bernstein, Stephen C. Woods, and Randy J. Seeley. Central infusions of leptin and GLP-1-(7–36) amide differentially stimulate c-FLI in the rat brain. *Am. J. Physiol.* 271 (Regulatory Integrative Comp. Physiol. 40): R1096–R1100, 1996.—Recently, glucagon-like peptide-1-(7–36) amide (GLP-1) and leptin have been implicated in the regulation of food intake. In the present study, we compared the effects of third ventricular administration (i3vt) of leptin (3.5 µg) and GLP-1 (10.0 µg) on short-term food intake and c-Fos-like immunoreactivity (c-FLI) in hypothalamic, limbic, and hindbrain areas in the rat. Relative to controls, infusion of leptin or GLP-1 (3 h before lights off) significantly reduced food intake over the first 2 h in the dark phase (53 and 63%, respectively). In different rats, infusion of leptin or GLP-1 elevated c-FLI in the paraventricular hypothalamus and central amygdala. Furthermore, leptin selectively elevated c-FLI in the dorsomedial hypothalamus, whereas GLP-1 selectively elevated c-FLI in the nucleus of the solitary tract, area postrema, lateral parabrachial nucleus, and arcuate hypothalamic nucleus. The fact that most of the c-FLI after leptin or GLP-1 administration was observed in separate regions within the central nervous system (CNS) suggests different roles for leptin and GLP-1 in the CNS regulation of food intake and body weight.

food intake; body weight; hypothalamus; amygdala; caudal brain stem

FOOD INTAKE AND BODY WEIGHT are regulated at the level of the central nervous system (CNS). Recently, two peptides, leptin, secreted from white adipose tissue (16, 31), and glucagon-like peptide-1-(7–36) amide (GLP-1), secreted from the distal portion of the gut (12), have been implicated in the CNS control of food intake and body weight. Circulating (2, 15) and central (22) levels of leptin correlate well with body adiposity, and central as well as peripheral administration of synthetic leptin reduces food intake and body weight (1, 5, 19, 29). GLP-1 is an incretin factor (8, 17), secreted from the L cells when nutrients enter the gastrointestinal tract (7, 10, 20). GLP-1 is also secreted by neurons in the CNS (9, 11, 25), and administration of GLP-1 into the third ventricle (i3vt) of rats reduces food intake (27). Given

the different physiological characteristics of the two peptides, we hypothesized that the reductions in food intake after i3vt leptin and GLP-1 administration represent different mechanisms and sites of action. Therefore, we chose doses of each peptide that cause similar reductions of food intake and then used c-Fos immunohistochemistry to assess increases in neural activity in limbic, hypothalamic, and hindbrain regions after administration of these doses in the rat.

MATERIALS AND METHODS

Animal preparation. Male Long-Evans rats weighing between 300 and 400 g were individually housed in stainless steel hanging cages and maintained on a 12:12-h light-dark cycle. Pelleted chow and water were continuously available (except where noted). Under Equithesin (3.3 ml/kg ip) anesthesia, rats ($n = 30$) were implanted with 21-gauge stainless-steel cannulas (Plastics One, Roanoke, VA) aimed at the third ventricle. With bregma and lambda at the same vertical coordinate, cannulas were placed directly on the midline, 2.3 mm posterior to bregma and 7.8 mm ventral to dura (18), and fixed to the skull with anchor screws and dental acrylic. The cannulas were fitted with removable obturators that extended 0.5 mm beyond the tip of the guide cannula. After surgery, each rat was given 0.15 ml each of Chloromycetin (100 mg/ml sc) and gentamicin (40 mg/ml ip) prophylactically. One week later, cannula placements were confirmed by administration of 10 ng angiotensin II in saline. Animals that did not drink 5 ml of water within 60 min were not used ($n = 2$). Continuing rats were allowed to recover for at least an additional 2 wk, during which time they all had returned to above presurgical weights and were handled twice daily for adaptation to the experimental procedures.

Assessment of food consumption. On 2 experimental days, food was removed 3 h before lights off and rats ($n = 12$) were weighed. On the first experimental day, rats were divided into two groups matched for body weight ($n = 6$ each). One group was infused with synthetic cerebrospinal fluid (s-CSF) and the other was infused with GLP-1 (10.0 µg dissolved in s-CSF; American Peptide, Sunnyvale, CA). After 3 recovery days, three randomly assigned rats from each group were infused with leptin (3.5 µg dissolved in s-CSF). Leptin (at least 90% pure) was harvested from an expression system in which recombinant DNA of the human leptin gene was overexpressed in *Escherichia coli* (for details, see Ref. 1). For infusion, the obturator was removed and a 26-gauge infusion cannula connected to PE-20 infusion tubing was inserted into

the guide cannula. This infusion cannula extended 1.0 mm beyond the tip of the guide cannula. Solutions (3.5 μ l) were manually infused with a Hamilton syringe over 60 s. Immediately after infusion, the obturator was replaced. Food was weighed and returned to the cage 1 h later, and intake was assessed hourly until 2 h into the dark period.

c-Fos-like immunoreactivity procedures. The remaining rats ($n = 16$) were infused during a 2-h window ending 3 h before lights off. Rats were divided into three groups matched for body weight, and animals in each group were infused with one of the three solutions used in the previous experiment (leptin, $n = 5$; GLP-1, $n = 5$; vehicle, $n = 6$). Two hours later, rats were anesthetized with Equithesin (3.3 ml/kg ip) and transcardially perfused with isotonic phosphate-buffered saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed and postfixed for ~ 24 h and then processed for c-Fos-like immunoreactivity (c-FLI). An additional nonimplanted group was handled like the other groups throughout the experiment and served as a control for stress-induced c-FLI ($n = 3$). Seventy-five-micrometer slices were cut from brains with a vibrotome. Coronal sections were taken from the forebrain. Horizontal slices were made from the hindbrain to allow visualization of the rostrocaudal extent of the nucleus of the solitary tract (NTS) and the parabrachial nucleus (PBN). Tissues were prepared for c-FLI according to methods described elsewhere (25). Camera lucida drawings of c-FLI-positive brain structures were prepared by an experimenter naive to group treatments. Particular care was taken to score different structures (randomly selected from left or right hemisphere) in the same plane. With all other structures, drawings were made in an area of ~ 0.38 mm² and were scored by blind raters who recorded the number and location of c-FLI nuclei. Scores across raters were averaged for statistical analyses.

Data analyses. Differences between the groups in food intake and c-FLI in response to drug infusion were analyzed with one-way analysis of variance (ANOVA). Separate ANOVAs were performed on data collected from each brain structure. When significant differences were found, post hoc analyses were conducted using Student's *t*-tests. In all cases, $P < 0.05$ (two-tailed) indicated statistical significance.

RESULTS

Effects of leptin and GLP-1 on food consumption. Cumulative food intake in rats over a 4-h period (2 h in the dark phase) after i3vt infusion of leptin, GLP-1, or s-CSF was 0.7 ± 0.2 , 0.5 ± 0.1 , and 1.4 ± 0.2 g/100 g body wt, respectively. ANOVA revealed a significant drug effect [$F(2,15) = 9.67$]. Post hoc tests revealed that, whereas both leptin and GLP-1 produce significant reductions in food intake compared with the s-CSF

group, food intakes were not different between leptin and GLP-1.

Effects of leptin and GLP-1 on c-FLI. Data from c-FLI-positive forebrain and hindbrain structures are depicted in Fig. 1, A and B, respectively. Representative photomicrographs of the paraventricular (PVH), the dorsomedial (DMH), and the arcuate (Arc) regions of the hypothalamus, the central nucleus of the amygdala (CeA), and the NTS are depicted in Fig. 2. Relative to the s-CSF and noninfused groups, both leptin and GLP-1 caused increased c-FLI in the PVH and the CeA. Leptin induced increased c-FLI in the DMH, whereas GLP-1 induced c-FLI in the Arc, the area postrema (AP), NTS, and lateral PBN (l-PBN). ANOVAs revealed that there were significant drug effects on c-FLI in PVH [$F(3,15) = 11.05$], DMH [$F(3,15) = 21.10$], Arc [$F(3,15) = 6.86$], and CeA [$F(3,15) = 56.44$] in the forebrain. Post hoc tests comparing control groups (s-CSF and noninfused) to drug-treated groups revealed that both leptin and GLP-1 caused increased c-FLI in the PVH and the CeA, that only leptin increased c-FLI in the DMH, and that only GLP-1 increased c-FLI in the Arc. c-FLI in the CeA was significantly greater after GLP-1 than after leptin. In the hindbrain, ANOVAs revealed significant drug effects on c-FLI in AP [$F(3,15) = 61.83$], NTS [$F(3,15) = 69.41$], and l-PBN [$F(3,15) = 78.10$]. Post hoc tests comparing control groups to drug-treated groups indicated that GLP-1 significantly increased c-FLI in the AP, NTS, and l-PBN.

DISCUSSION

The present results confirm that i3vt administration of leptin and GLP-1 each causes a reduction in food intake over 2 h in the dark phase. Thus the results are consistent with the recent reports that leptin (1, 5, 19, 29) and GLP-1 (27) suppress food intake by actions directly in the CNS. Importantly for consideration of the c-FLI data, the selected doses of leptin (3.5 μ g) and GLP-1 (10.0 μ g) reduced food intake comparably (53 and 63%, respectively).

The present results demonstrate for the first time that i3vt administrations of leptin and GLP-1 elevate c-FLI in distinct areas in the CNS. Only GLP-1 caused elevation of c-FLI in hindbrain regions (NTS, l-PBN, and AP) and the Arc, and only leptin caused elevation of c-FLI in the DMH. Furthermore, both leptin and GLP-1 caused elevation of c-FLI in the PVH and the CeA. The

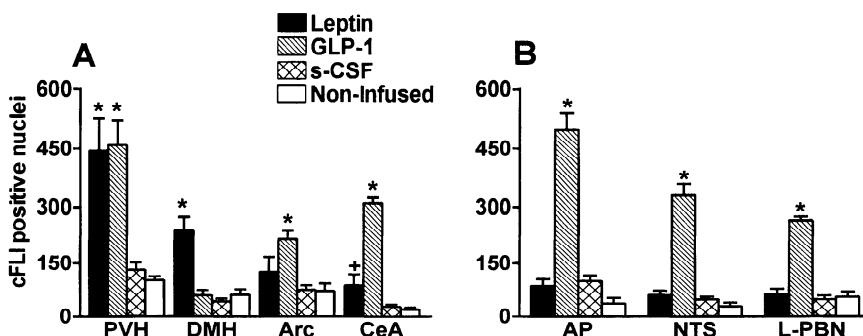


Fig. 1. Mean (\pm SE) c-Fos-like immunoreactivity (c-FLI)-positive nuclei in forebrain structures (A): the paraventricular (PVH), the dorsomedial (DMH), and the arcuate (Arc) nuclei of the hypothalamus and the central amygdala (CeA) after infusion of leptin, glucagon-like peptide-1-(7–36) amide (GLP-1), or synthetic cerebrospinal fluid. B: c-FLI-positive nuclei in the hindbrain structures: the area postrema (AP), the nucleus of the solitary tract (NTS), and the lateral parabrachial nucleus (l-PBN). *Significant at $P < 0.001$ relative to controls (s-CSF or noninfused); + significant at $P < 0.01$ relative to controls.

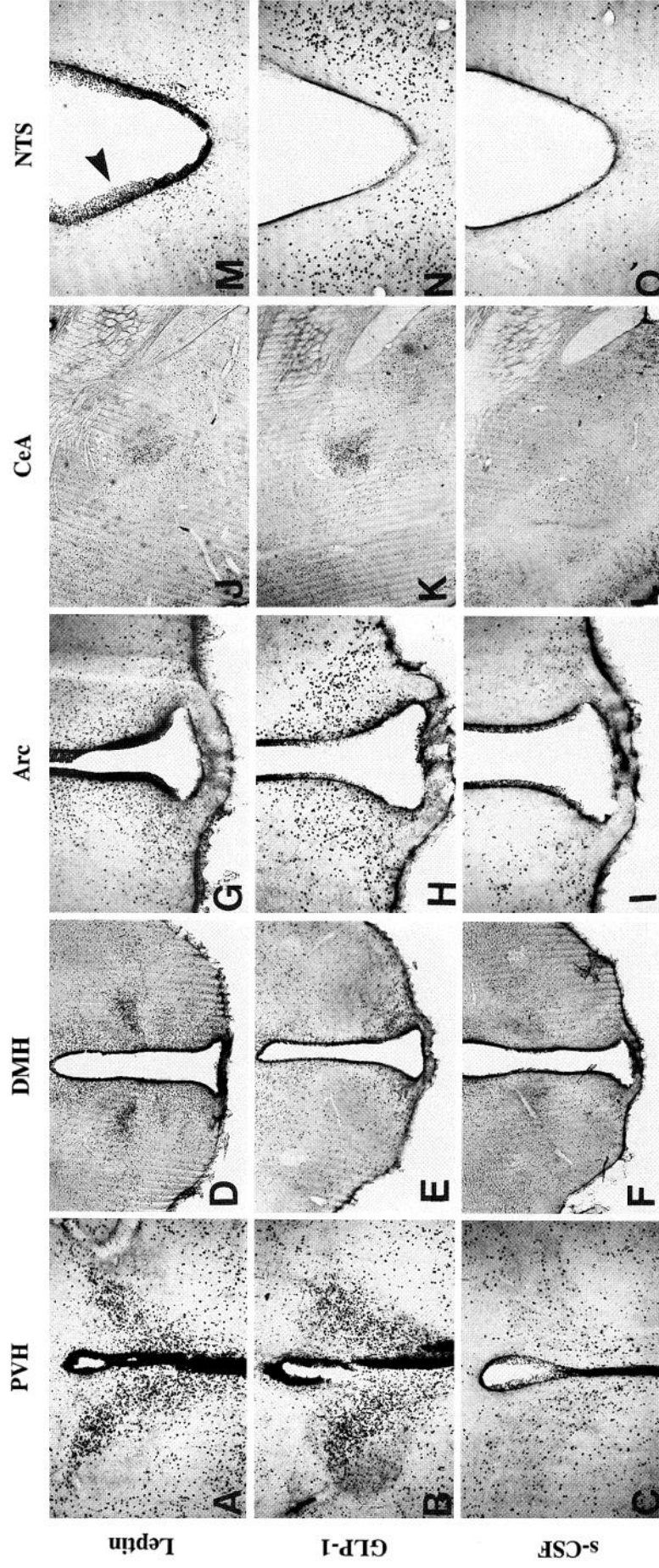


Fig. 2. Representative photomicrographs of the c-Fli-positive nuclei in PVH (A-C), DMH (D-F), Arc (G-I), CeA (J-L), and NTS (M-O). Animals were infused with either leptin, GLP-1, or s-CSF (top, middle, and bottom, respectively). Arrow in M indicates c-Fli-positive staining in the choroid plexus.

elevated c-FLI in the CeA, which mainly appeared in the lateral CeA (l-CeA), was more pronounced after GLP-1 than leptin. Similar overall c-FLI values were obtained in the PVH after administration of leptin and GLP-1. The localization of c-FLI within the PVH appeared to be, for the most part, in the medial parvocellular subdivision.

The receptor for leptin has recently been cloned, and preliminary reports suggest that central leptin receptors are localized within the hypothalamus and the choroid plexus (26). This is consistent with the elevated c-FLI in the PVH and DMH as well as the dense c-FLI along the ventricular walls after leptin administration (see Fig. 2M; arrow). So, although c-FLI in a region does not necessarily imply that there is a direct action of the treatment on that region of the CNS, the present data are consistent with a receptor-mediated role for leptin in the integration of food intake and body weight in hypothalamus and possibly limbic regions as well.

Messenger RNA transcripts encoding for proglucagon precursors have been found in the NTS (6), and GLP-1 binding sites have been localized in many brain areas, including those that expressed GLP-1-induced c-FLI in the present experiment (4, 24, 28). Thus it is possible that GLP-1 is involved in relaying visceral information at the level of the NTS and that secondary ascending fiber systems projecting from the NTS activate the c-FLI-positive areas identified in the present study (14). Because solutions administered i3vt readily gain access to the hindbrain (21), it is possible that i3vt administration of GLP-1 stimulated neural activity at all levels of this ascending pathway in the hindbrain. The endogenous signals that are responsible for stimulating central GLP-1 secretion are not known to date. An obvious candidate is peripheral GLP-1, which may modulate feeding behavior by acting directly on GLP-1 receptors in the CNS (8) or, hypothetically, via GLP-1 receptors on vagal afferents.

An analogy may be drawn between the effect of GLP-1 in the present study and that of peripheral administration of the satiety hormone (30), cholecystokinin octapeptide (CCK-8), in other studies. Both peptides elicit elevated c-FLI in the NTS, AP, l-PBN, PVN, and l-CeA (present results, 3, 13, 27). Although some differences exist between c-FLI after peripheral administration of CCK-8 (supraoptic nucleus) and i3vt administration of GLP-1 (Arc), the present data are consistent with the possibility that GLP-1 and CCK influence food intake similarly. Alternatively, i3vt GLP-1 may reduce food intake via a nonspecific effect because the pattern of c-FLI after i3vt GLP-1 (NTS, AP, l-PBN, CeA) also resembles the pattern that is expressed after administration of the emetic agent, lithium chloride (25).

Perspectives

The present results demonstrate that two treatments that reduce food intake equivalently can induce considerably different patterns of neuronal activation. Such a result implies that the function of these two endogenous peptides in the control of food intake may also be quite different. Because the levels of leptin in the

plasma appear not to change acutely with the consumption of meals (2, 15), leptin would appear not to be a good candidate for regulating the termination of ingestive bouts. GLP-1 levels in the periphery, however, are elevated in response to food consumption (8, 10), and consequently the possibility remains that peripheral or central GLP-1 may be involved in meal termination. Additionally, whereas leptin administration has a potent inhibitory effect on long-term food intake and body weight (1, 5, 19, 29), there are no data of which we are aware that GLP-1 administration can inhibit long-term food intake or body weight. This supports the hypothesis that, whereas leptin is a body weight regulatory factor, GLP-1 may inhibit food intake only in the short term. The different patterns of c-FLI may reflect these different roles. Body weight regulatory factors, like leptin, activate primarily forebrain (e.g., hypothalamic and limbic) circuits, whereas compounds that regulate meal termination, such as CCK, bombesin-like peptides, and GLP-1 activate a number of caudal brain stem as well as forebrain circuits.

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